# *Semi-Annual Status Report*

## *NASA Grant SC NsG 300-63*

*30 April 1965*




## *Oklahoma City University*

*Oklahoma City, Oklahoma*

# TABLE OF CONTENTS

## ABSTRACT

Evidence collected to date indicate exposure of rats for four weeks to a high oxygen-low pressure environment (100% $O_2$, 5 psia) produces symptoms of marginal oxygen toxicity. After more prolonged exposure (eight and twelve weeks), many of the parameters which were observed earlier to be abnormal tend to return towards the levels found in the comparable control animals, although some did not. Interestingly, a few of the parameters were found to be abnormal in the opposite direction after twelve weeks of exposure.

We conclude that the response of CoA was as expected from a marginal oxygen toxicity. In the early stages of the experiment, CoA was destroyed by a high cellular level of oxygen. On longer exposure, the oxygen tension at the cellular level was decreased and CoA concentration was allowed to return to normal. Several minor changes appeared to contribute to the decreased cellular oxygen tension. The hematocrit values of the experimental animals decreased while hemoglobin suffered parallel decreases. Small increases in methemoglobin were observed in the blood of these animals though, again, glucose-6-phosphate

dehydrogenase activity roughly paralleled the changes in numbers of red cells.

The $CO_2$ concentration in the blood was always higher throughout the twelve weeks of exposure. This could also contribute to a decreased oxygen transport capacity of blood. The relationship of the blood $CO_2$ level to $CO_2$ expiration is discussed. Perhaps related to this is the dramatic increase in the size of the heart after twelve weeks of exposure.

Metabolically, the heart and kidney apparently had more effective homeostatic mechanisms than did the liver. In both the heart and kidney, $C^{14}$-acetate incorporation into glycogen, which was reduced after four weeks of exposure, was identical to that in the control animals after twelve weeks of exposure. In the liver, $C^{14}$ incorporation was much greater after twelve weeks.

Detailed analysis of the lipids in the heart and liver were completed on animals exposed to the test environment for four weeks and showed that in quantitative terms the tissues of the experimental animals contain significantly more cholesterol. With respect to $C^{14}$-acetate incorporation, the liver incorporates less into triglycerides and almost none into unesterified fatty

acids but incorporates a great deal into the mono- and diglyceride fraction. In the heart, the major difference in $C^{14}$ incorporation was the definite increased incorporation into sterols (almost entirely cholesterol), both free and esterified. Plausible interpretations of these data are discussed. Although urinary ketone body concentration remained unchanged, the radioactivity in the carboxyl carbon of acetoacetate was increased at four weeks in the experimental animals and was even more affected by the twelfth week.

During this period, a manuscript entitled "Effects of Discontinuous Exposure to a High Oxygen-Low Pressure Environment on Rats" was prepared for submission for publication in AEROSPACE MEDICINE.

# I. INTRODUCTION

The previous status reports have suggested that the alterations in metabolism demonstrated in animals exposed to an environment essentially made up of oxygen at 5 psia were related to a marginal hyperoxic situation at the cell level but that the animals tend to adapt to this environment after exposure to it for 12 weeks. The work presented here represents an attempt to verify the relative oxidative nature at the cellular level and the extent as well as the nature of the adaptation occurring.

This work is a continuation of the studies on the effects of the experimental environment on the turnover rates of metabolic pools after 4 and 12 weeks of exposure and additionally contains evidence of the oxidative state of the tissues in another experiment in which animals were sacrificed after living in the test environment for 4, 8 or 12 weeks. The latter experiment included studies of the $C^{14}O_2$ expiration and ketone body metabolism.

Essentially, it appears that many of the metabolic disturbances observable after 4 weeks tended to return towards normal

after exposure for 12 weeks. However, this was not the case with several parameters. The hearts of the experimental animals were much larger and the blood $pO_2$ and A-V difference in $pO_2$ was considerably less than in the comparable control animals. Additionally, differences in the radioactivity incorporation into acetoacetate observed after 4 weeks become progressively greater after 8 and 12 weeks.

Measurement of blood gases indicate that after 4 weeks of exposure the experimental animals were suffering from uncompensated respiratory acidosis.

## II. EXPERIMENTAL

During the period covered by this report, a detailed analysis of the turnover rates of metabolic pools in various tissues was initiated for animals exposed to the experimental environment for 4 and 12 weeks. Lipids were fractionated by thin-layer chromatography using n-hexane, diethylether and acetic acid in a ratio of 90:10:1 as the development system. The resolved lipid fractions were detected with the aid of iodine vapors, scraped into counting vials and counted in the liquid scintillation spectrophotometer in the presence of 20 ml of a dioxane-POPOP scintillation solution. In cases where quenching was indicated the addition of an internal standard was made using $10^5$ dpm of $C^{14}$-acetate.

An attempt was made to quantitate the amount of lipid on each spot of the chromatogram. The developed plates were sprayed with a 10% $H_2SO_4$ solution and heated in a covered hot plate at 85°C for 30 minutes. The charred plates were placed on a Photovolt TLC Densitometer to quantitate the free sterols (essentially cholesterol). Unfortunately, it was observed that the degree of unsaturation in the fatty acids greatly

influences the density of char observed on the plates. Since we have already reported an alteration in the ratio of saturated to unsaturated fatty acids in the experimental animals, this method of quantitation, although reasonably simple and quite reproducable, was not very accurate in the case of fractions that contained fatty acids. Thus, we were unable to quantitate the phospholipids; mono-, di- and triglycerides; sterol esters and unesterified fatty acids with this technique.

Following saponification of an aliquot of the unfractionated lipid, the fatty acids were separated as the methyl esters on a 7 1/2 ft., 10% diethyleneglycolsuccinate column in a gas-liquid chromatograph equipped with a four filament katharometer. The effluent fractions were collected in a glass tube which was cooled in a dry ice-acetone bath. Identification was made by comparison of the retention times of the recorded fatty acids with those of known standards.

Glycogen determinations were reported in our last status report for animals exposed to the test environment for 4 weeks. In general the same procedure was used to precipitate the glycogen in 12 week exposed animals. However, in order to obtain

a more accurate measurement of the amount of carbohydrate present, an aliquot of the lipid extracted solubilized homogenate was hydrolysed in 10% $H_2SO_4$ on a boiling water bath for 1.5 hours and the glucose concentration was determined colorimetrically by the copper reduction technique (1).

A second long-term experiment was concluded during this period. Twenty 325 gm rats were placed in the experimental and also in the control chamber. After exposure to the test environment for 4 and 8 weeks, three animals were returned to the laboratory environment, injected with acetate-1-$C^{14}$ at the rate of 700 μc/kg of body weight, and placed in the metabolic chamber for 8 hours. During this period, the expired $CO_2$ was trapped and measured as previously reported for the 12 week exposed animals in the October 1964 status report. Thus, this portion of the experiment completes the accumulation of comparable $CO_2$ expiration data for animals exposed to the test environment for 4, 8 and 12 weeks.

Additionally, this second long-term experiment was designed to remove animals after 4, 8 and 12 weeks to determine arterial and venous $pO_2$ and $pCO_2$. Five experimental

and five control animals were sacrificed for this experiment after 4 and 8 weeks of exposure and four animals of each group were sacrificed after being exposed for 12 weeks to the test environment.

Although it was recognized that the use of any anaesthetic will tend to reduce the oxygen carrying capacity of blood, it was impractical to insert arterial and venous cannulae in animals in our chamber system. Therefore, on the advice of Dr. B. E. Welch, we injected Sodium Pentothal (Abbott) into the tail vein of each rat at the rate of 12 mg/kg of body weight. This technique anaethesized the animal in about 3 seconds. The body cavity was opened and heparinized blood was obtained from the hepatic vein and the abdominal aorta as anaerobically as possible. The samples were stored in the refrigerator under mineral oil and the $pO_2$-$pCO_2$ measurements were done in duplicate on the Van Slyke apparatus that same day.

Routine physiological measurements were also made on these animals as well as glucose-6-phosphate dehydrogenase and coenzyme A determinations as outlined in our last two

status reports. Hemoglobin and methemoglobin was determined by a routine clinical technique (2).

Ketone bodies, excluding $\alpha$-hydroxybutyrate were measured (3) in the urine of rats subjected to the experimental environment for 12 weeks. In addition, radioactivity in urinary acetoacetate and acetone was determined. The latter measurement was made by adding carrier acetoacetate, urine, and acid to a reaction flask that was flushed with nitrogen. After dissolved carbon dioxide was removed by this procedure, the reaction flask was heated to decarboxylate the acetoacetate. The carboxyl carbon was trapped as carbon dioxide in sodium hydroxide, precipitated as barium carbonate and counted in a liquid scintillation counter (4). The acetone was trapped as the 4-phenylsemicarbazone, recrystallized and assayed for radioactivity in a liquid scintillation counter.

## III. PHYSIOLOGICAL RESPONSE TO EXPERIMENTAL ENVIRONMENT

### a. General

In the second 12 week experiment, the animals were housed in the chambers described in our April 1964 status report. The water consumption data (Table 1) were somewhat lower than we previously reported. This was apparently due to a change in the electrodes of the water control system which prevented wastage of water. Also, the chambers were maintained at a slightly lower temperature during this experiment. Notwithstanding, the relative relationship between experimental and control

TABLE 1

WATER CONSUMPTION

(ml/animal/day)

| Period | Control | Experimental | Ratio Expt/Cont |
|---|---|---|---|
| 1st 4 weeks | 35.9 | 38.5 | 1.07 |
| 2nd 4 weeks | 35.7 | 40.4 | 1.13 |
| 3rd 4 weeks | 40.3 | 43.4 | 1.08 |
| All 12 weeks | 37.3 | 40.7 | 1.08 |

animal water consumption is in good agreement with our previous data. No significant differences in food consumption were observed during the entire 12 week experiment.

The rate at which oxygen entered the chamber averaged 8.9 l/min (STP) and the by-pass was set at 6.8 l/min. The altitude was held at $\pm$ 58 feet. The average barometric pressure in the control chamber was 741.6 mm Hg, and the average temperature in the chambers was 21.7°C.

b. Body and tissue weights.

In the October 1964 status report, it was shown that there was no difference in body weight after 4 weeks of exposure but after 8 and 12 weeks of exposure, the experimental animals were somewhat heavier. In the challenge experiment (Table 2) the results were the same in qualitative terms. Statistically the differences were never significant in this experiment, but the sample size was about 25% of that used in the previous experiment. It appears, therefore, that there is a consistent tendency for the experimental animals to gain more weight than the control animals. Previously we suggested that this might be attributed to edema. However, the water consumption data (Table 1) show

TABLE 2

BODY AND TISSUE WEIGHT AS INFLUENCED BY CONTINUOUS EXPOSURE TO THE EXPERIMENTAL ENVIRONMENT

| | Exposure Time Weeks | Tissue Weight (gm) Control | Exptl | P< | Tissue Weight as % of Body Weight Control | Exptl | P< |
|---|---|---|---|---|---|---|---|
| Whole Body | 1 week prior to start of experiment | 326 | 324 | 0.6 | | | |
| | 4 | 423 | 415 | 0.5 | | | |
| | 8 | 455 | 457 | 0.9 | | | |
| | 12 | 475 | 496 | 0.4 | | | |
| Liver Weight | 4 | 13.4 | 13.7 | 0.7 | 3.17 | 3.30 | 0.4* |
| | 8 | 14.7 | 14.4 | 0.7 | 3.18 | 3.15 | 0.7* |
| | 12 | 15.3 | 16.3 | 0.4 | 3.24 | 3.28 | 0.8* |
| Kidney Weight | 4 | 2.81 | 3.07 | 0.01 | 0.67 | 0.73 | 0.05* |
| | 8 | 3.14 | 3.12 | 0.1 | 0.68 | 0.69 | 0.8* |
| | 12 | 3.08 | 3.23 | 0.6 | 0.65 | 0.66 | 0.9* |
| Heart Weight | 4 | 1.38 | 1.39 | 0.9 | 0.33 | 0.33 | 1.0* |
| | 8 | 1.42 | 1.41 | 1.0 | 0.31 | 0.31 | 0.8* |
| | 12 | 1.48 | 1.74 | 0.01 | 0.31 | 0.35 | 0.01* |
| Brain Weight | 4 | 1.92 | 2.05 | .01 | 0.46 | 0.49 | 0.2* |
| | 8 | 2.00 | 2.05 | 0.3 | 0.44 | 0.44 | 0.9* |
| | 12 | 2.02 | 2.04 | 0.7 | 0.43 | 0.41 | 0.5* |
| Lung Weight | 4 | 1.86 | 2.01 | 0.4 | 0.44 | 0.51 | 0.3* |
| | 8 | 1.84 | 2.11 | 0.2 | 0.40 | 0.53 | 0.1* |
| | 12 | 1.95 | 2.06 | 0.7 | 0.41 | 0.43 | 1.0* |

*Statistical analyses of tissue weight as % of body weight done on only 3 experimental animals.

that the experimental animals "consumed" about 9% more water than the control and all of this can be accounted for through the greater evaporation rate in the experimental chambers. Carcass analyses which should be completed this summer will hopefully clarify this question.

In agreement with our last status report, the livers of the experimental animals tended to be heavier after 4 and 12 weeks of exposure with a suggestion that this may not be true at 8 weeks. However, in this experiment the difference was significant after 4 weeks of exposure only.

The pattern with respect to the heart was exactly in agreement with our last report in that it tended to remain unaffected through 8 weeks of exposure but was very significantly larger in the experimental animals after 12 weeks of exposure. This applied both to the tissue weight and when expressed as a percent of body weight.

Again, the brains of the two groups of animals were not appreciably different after 8 or 12 weeks of exposure, particularly when expressed as a percent of body weight. However, after 4 weeks of exposure, the brains of the experimental animals

were significantly heavier.

The raw data on the lung weights were not as boldly different as in the last experiment but one notes a trend toward a greater weight. It therefore seems desirable now to study the lipids involved in the lung surfactant materials, especially in view of the $pO_2$ data presented later in this section. We are presently pursuing this study.

When viewed in the aggregate, the data are, in general, consistent with the concept that these animals are adapting to the experimental environment with increased time of exposure. The major exception to this is heart weight. This tissue appears to resist alteration during the first 8 weeks but by 12 weeks has been considerably affected. The implications of this are discussed more fully in the following section.

c. Blood gases.

The data point to a very interesting and complex picture (Table 3). It is apparent that the $pCO_2$ is always higher in the experimental animals. Carbonic anhydrase would convert $CO_2$ to $H_2CO_3$ and tend to lower the blood pH. As reported in our April 1964 status report, after 4 weeks exposure, the

TABLE 3

BLOOD GASES
(Volumes %)

| Time of Exposure | Blood Gas | Control Arterial | Control Venous | Experimental Arterial | Experimental Venous | P< Arterial | P< Venous |
|---|---|---|---|---|---|---|---|
| 4 wks | $O_2$ | 14.5 | 11.2 | 15.7 | 11.9 | .02 | .5 |
| | $CO_2$ | 38.2 | 46.5 | 44.2 | 53.3 | .01 | .01 |
| 8 wks | $O_2$ | 16.2 | 12.4 | 14.4 | 11.0 | .1 | .05 |
| | $CO_2$ | 42.0 | 46.4 | 45.8 | 56.2 | .4 | .01 |
| 12 wks | $O_2$ | 16.8 | 12.5 | 14.7 | 12.5 | .1 | .9 |
| | $CO_2$ | 35.1 | 43.8 | 45.8 | 57.2 | .01 | .01 |

TABLE 4

A-V DIFFERENCES IN VOLUME PERCENT OF BLOOD $O_2$

| Time of Exposure | Control | Experimental | P< |
|---|---|---|---|
| 4 wks | 3.3 | 3.8 | .7 |
| 8 wks | 3.7 | 3.5 | .9 |
| 12 wks | 4.3 | 2.3 | .01 |

control animal blood pH was 7.49 while pH in the experimental animals was 7.36 ($P < 0.001$).

$pO_2$ was greater in the 4-week exposed experimental animals on both the arterial and venous samples. The A-V difference was also greater by about 15%. This was as one might expect if this environment resulted in a marginal hyperoxic situation. But it is interesting that the control animals showed a consistent increase in both arterial and venous $pO_2$ with increase in age while the experimental animals showed a tendency to decline with time so that the arterial values became lower than comparable control values.

Twelve weeks after the experiment was begun, the venous $pO_2$ values were the same in both groups of animals. This might indicate again a homeostatic adaptation. But because of the decreasing arterial $pO_2$ in the experimental animals the A-V difference (Table 4) are very close at 8 weeks and greatly reduced in the case of the experimental animals after 12 weeks of exposure.

The blood pH and $pCO_2$ data suggest that after 4 weeks of exposure to the experimental environment the animals suffered

from an uncompensated respiratory acidosis (5). It is hoped that a clearer interpretation of this data will be derived this summer.

## IV. TISSUE OXIDATION

### a. Coenzyme A and Sulfhydryl Containing Enzymes

Previous experiments, in which oxygen at high pressure caused sever oxygen toxicity symptoms, demonstrated that sulfhydryl containing enzymes and coenzymes were particularly susceptible to oxygen damage (6). After 4 weeks exposure to the space-cabin environment, we found there was a significant decrease in total free sulfhydryl groups of brain and liver. Since this measurement did not specify which materials were being removed, further investigations were directed toward specific systems. The investigation of coenzyme A concentration in various tissues of rats was therefore initiated.

Brain tissue from experimental rats was compared to that from control rats maintained in similar chambers but in a normal atmosphere. In addition, these were compared to animals taken from our regular animal quarters. It was found that brain tissue in both types of control animals showed a remarkable uniformity. The experimental animals however, showed a regular, almost linear decrease in the concentration of CoA over the first four weeks of exposure (Fig. 1). The rate of

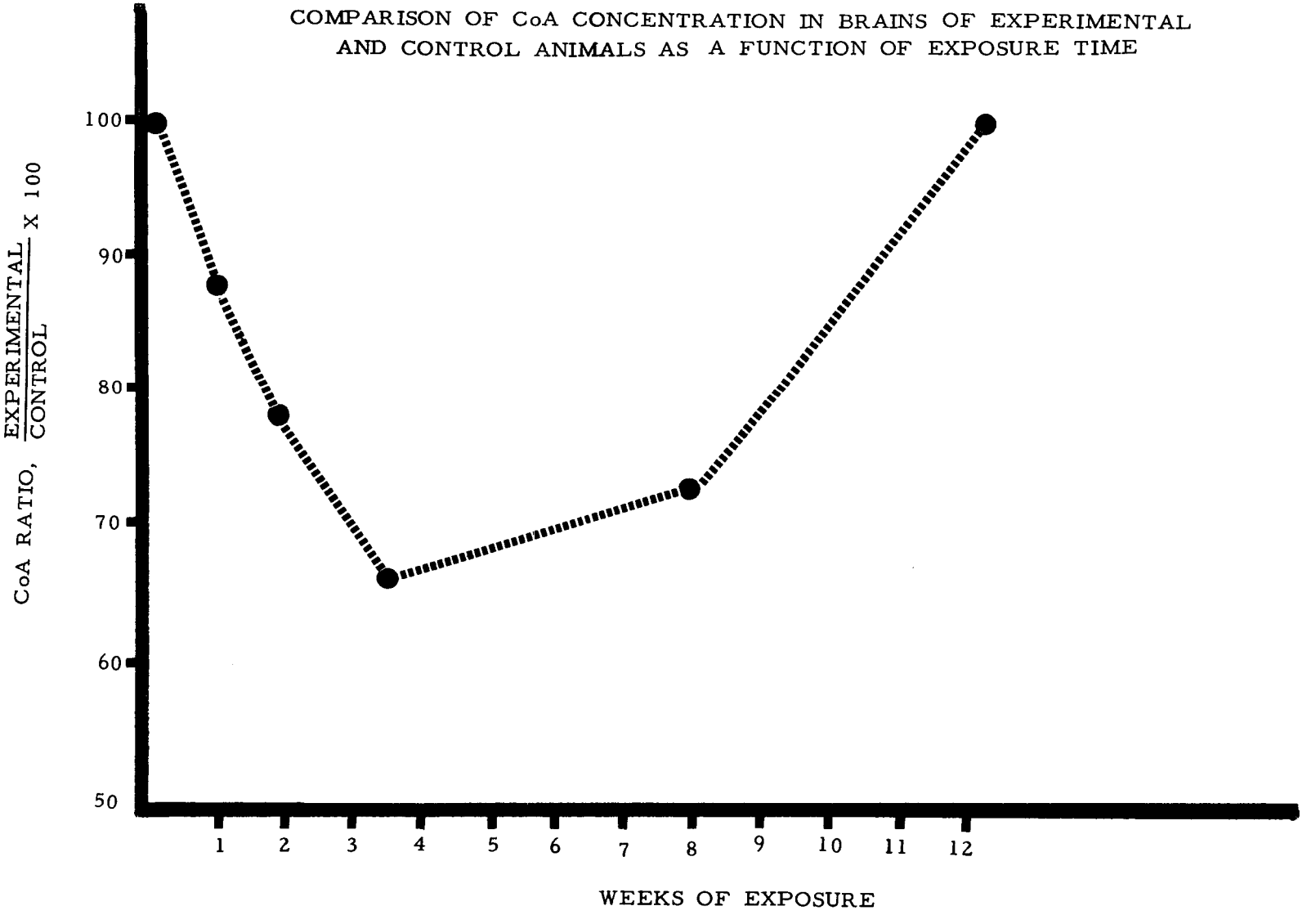
COMPARISON OF CoA CONCENTRATION IN BRAINS OF EXPERIMENTAL AND CONTROL ANIMALS AS A FUNCTION OF EXPOSURE TIME
CoA RATIO, EXPERIMENTAL / CONTROL X 100
100
90
80
70
60
50
1
2
3
4
5
6
7
8
9
10
11
12
WEEKS OF EXPOSURE

Figure 1

decrease varied somewhat from experiment to experiment but remained essentially linear in each instance. The greatest decreases were observed in two sets of animals deliberately subjected to the stress of a return to normal atmospheric conditions for 15 minutes every third and seventh day. Nevertheless, these animals differed from the usual experimental animals by only a few percent. Perhaps small variations of temperature or other ill-defined parameters may alter somewhat the absolute metabolic changes observed within an experiment, but the pattern of metabolic change is consistent among successive experiments.

Prolonged exposure to the space environment did not result in further adverse effects to the animal. After eight weeks exposure it was found that the CoA concentration was at a slightly higher level than that attained after four weeks exposure. After twelve weeks exposure, no significant difference between experimental and control animals could be detected. Similar results were observed in liver, kidney and heart tissue. Another prolonged exposure experiment has just been completed but data are not yet available for comparison with those previously completed.

b. Methemoglobin

Since the response to the experimental environment initially resembles oxygen toxicity at the cellular level, a possible means of adaptation would involve reducing the concentration of oxygen penetrating to that level. One method by which this could be done would be to reduce the oxygen transferring capacity of red blood cells (RBC). A minor conversion of hemoglobin to methemoglobin would have a marked effect on the oxygen transferring capacity of RBC.

The concentration of methemoglobin was measured in the blood of control and experimental animals after exposures of 4, 8 and 12 weeks. While the concentration of methemoglobin corresponded to a conversion of less than 5% of the total hemoglobin in the experimental animals, it was an increase over that in the control animals. While such a loss of hemoglobin and consequent gain in methemoglobin would not be considered damaging, it should have a measurable effect on the oxygen transferring capacity of blood. This seems to be borne out by our blood $pO_2$ data.

c. Glucose-6-phosphate Dehydrogenase

Since the concentration of methemoglobin in RBC is usually controlled by the activity of G-6-PDH, the number of units of this enzymatic activity per ml of blood was determined. A decrease in the activity of this enzyme in the blood of experimental animals was observed. The average decrease in enzyme activity was 87 percent of controls at 4 weeks, 80 percent at eight weeks and 87 percent at 12 weeks though this difference was statistically significant only at the eight weeks measurement. Based upon the units of activity per red cell (corrected for variation in hematocrit), the above measurements would suggest that there was no appreciable change between control and experimental animals.

d. Hematocrits

A measure of experimental and control hematocrits at the fourth, eighth and twelfth week of the experiment is reported in Table 5. While changes in hematocrit shown by the experimental animals are not large (approximately 11% at 4 weeks, 7% at 8 weeks and 7% at twelve weeks) and could not be construed as dangerous to the animal, they are significantly different from the controls. This degree of change has appeared in our experiments donsistently.

TABLE 5

HEMATOCRIT VALUES

| Period of Exposure | Animals | Mean Hematocrit Value | Level of Significance | $\frac{\text{Experimental}}{\text{Control}} \times 100$ |
|---|---|---|---|---|
| 4 weeks | Experimental | 39.6 | $P < 0.01$ | 89 |
| 4 weeks | Control | 44.3 | | |
| 8 weeks | Experimental | 40.7 | $0.02 < P < 0.05$ | 93 |
| 8 weeks | Control | 44.9 | | |
| 12 weeks | Experimental | 40.6 | $P < 0.01$ | 93 |
| 12 weeks | Control | 43.4 | | |

The fact that the hematocrit value rises somewhat at the 8th and 12th week over that at the 4th week but remains low, tends to support our hypothesis of adaptation. Since over-all changes are marginal, perhaps only marginal adaptive measures are required.

e. Hemoglobin

Hemoglobin content of the blood was determined in experimental and control animals at 4, 8 and 12 weeks. The concentration of hemoglobin in experimental animals' blood expressed as a percentage of control was found to be 87% at 4 weeks, 94% at 8 weeks and 98% at 12 weeks. These differences were statistically significant at both 4 and 8 weeks. Since the hematocrit ratios were also reduced, it seems likely that this was a contributing factor in the above changes. Such small changes in the hemoglobin and methemoglobin concentration would not be expected to endanger the health of an animal and might even be considered "normal" by physiological standards. Nevertheless, the marginal oxygen toxicity detected during the first few weeks of exposure appears to be relieved by such minor alterations of the system, at least in several of the parameters examined.

# V. RESPIRATORY $C^{14}O_2$

Radioactivity in respiratory carbon dioxide has previously been reported to be depressed in experimental animals at 4 weeks (7) but is partially restored to normal by 12 weeks (8). Figure 2 shows a graph of radioactivity in expired $CO_2$ per 10 minutes versus time post injection after 8 weeks of exposure. The shape of the curve is very similar to that observed in 12 week animals (8), indicating that the recovery process begins before the eighth week of exposure. These data are not in conflict with our previous interpretation that the biochemical response of the animal to the experimental environment is a reduction in metabolic rate but the animal eventually adapts to this new environment. However, part of the delayed excretion of radioactive carbon dioxide may be attributable to dilution of $C^{14}O_2$ by the larger $CO_2$ pool present in the blood of experimental animals. (See Section III)

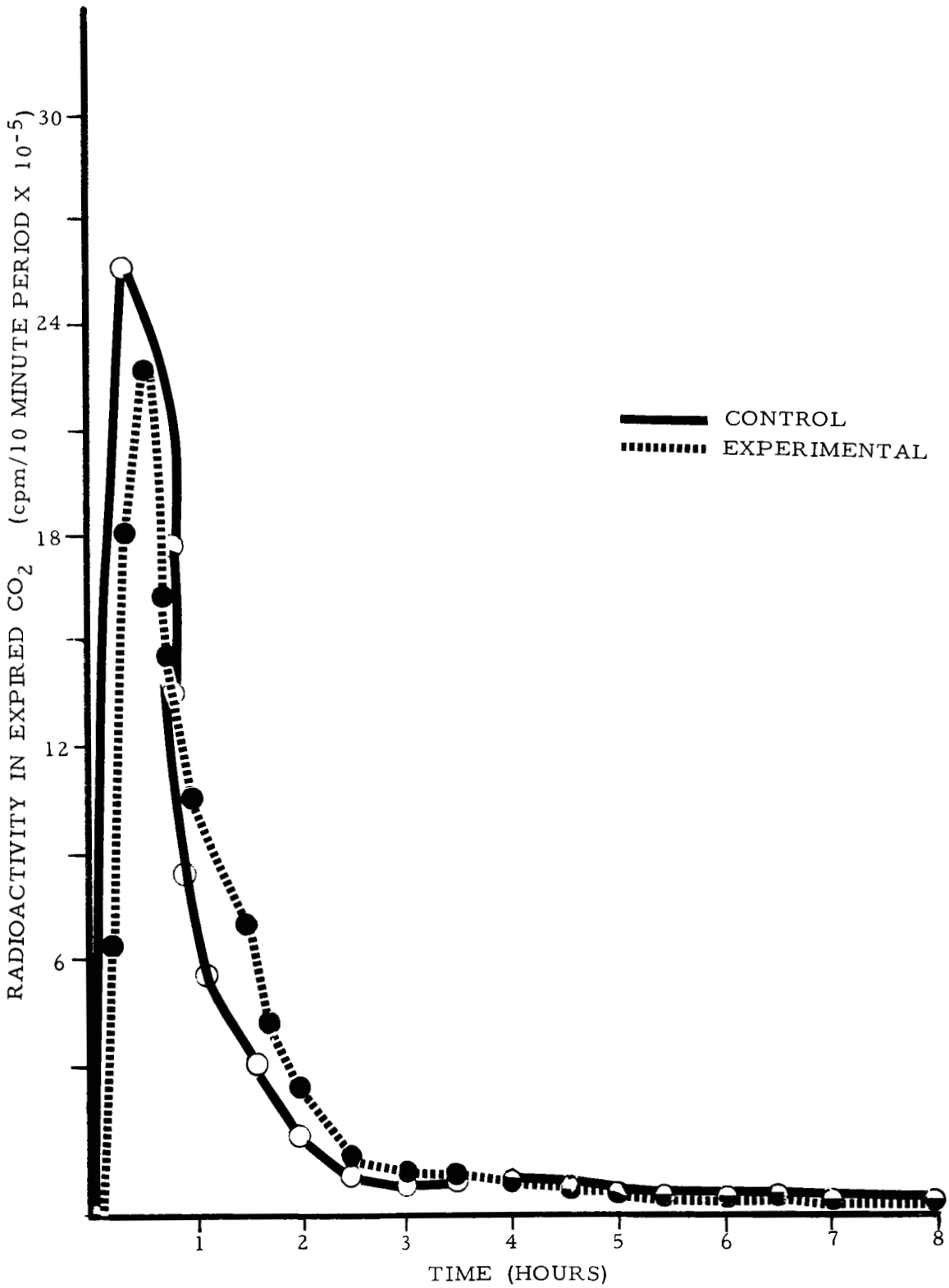


Figure 2. Expiration of Radioactive $CO_2$ During First Eight Hours after Injection of $C^{14}$-Acetate. (Duration of experiment - 8 weeks)

## VI. METABOLIC POOLS

### a. Lipid Content of Tissues

The lipid content of the liver, kidney and heart have been determined after 4, 8 and 12 weeks of exposure to the test environment (Table 6). The absolute values at 8 weeks are not as reliable as at 4 and 12 weeks because only three experimental and a like number of control animals were analyzed at 8 weeks.

Both groups of animals were understandably fatter after an experimental period of 12 weeks since they were more mature. As a point of interest, there were greater differences in lipid content of tissues due to age than due to the experimental environment. Further, it is interesting that the lipid content is the same in the heart of control and experimental animals after 4, 8 and 12 weeks even though heart size was considerably greater in the 12-week exposed experimental animals. The kidney in the experimental animals had less lipid after 4 and 8 weeks of exposure but had nearly as much lipid as the control animals after 12 weeks of exposure, again suggesting adaptation in the kidney.

TABLE 6

LIPID COMPOSITION OF TISSUES
(Expressed as % of Tissue Weight)

| Period of Exposure | Tissue | Control | Experimental |
|---|---|---|---|
| 4 weeks | Liver | 2.9 | 2.5 |
| | Kidney | 2.7 | 2.1 |
| | Heart | 2.5 | 2.5 |
| 8 weeks | Liver | 4.0 | 3.8 |
| | Kidney | 2.9 | 1.5 |
| | Heart | 1.8 | 1.8 |
| 12 weeks | Liver | 6.2 | 5.2 |
| | Kidney | 5.0 | 4.8 |
| | Heart | 3.8 | 3.7 |

TABLE 7

CHOLESTEROL CONTENT OF LIPID

| | Control (% of Lipid) | Experimental (% of Lipid) | Experimental (% of Control) |
|---|---|---|---|
| Liver | 2.74 | 3.48 | 127 |
| Kidney | 3.00 | 3.40 | 113 |

The liver of the experimental animals always had less lipid (14% less at 4 weeks and 16% less at 12 weeks) indicating that it was not as successful at adapting or that its homeostatic mechanisms were not as sensitive and effective as in the cases of the heart and kidney. Additional evidence has been gathered from animals that have been exposed for 4 weeks to the experimental environment in which both the heart and the liver contain more cholesterol but the difference between the experimental and control livers was twice as great as in the hearts (Table 7).

b. $C^{14}$ Incorporation into Glycogen

We have previously reported (8) that the glycogen content of the experimental animal livers was about 65% of that found in the comparable control animals. This held true after exposure to the test environment for 4 or 8 weeks. More recent work (Figure 3) shows that the rate of $C^{14}$-acetate incorporation into liver glycogen is markedly reduced by about 50%. This could account for the reduction in liver glycogen content. After exposure of the animals for 12 weeks to the experimental conditions, the specific activity of the liver

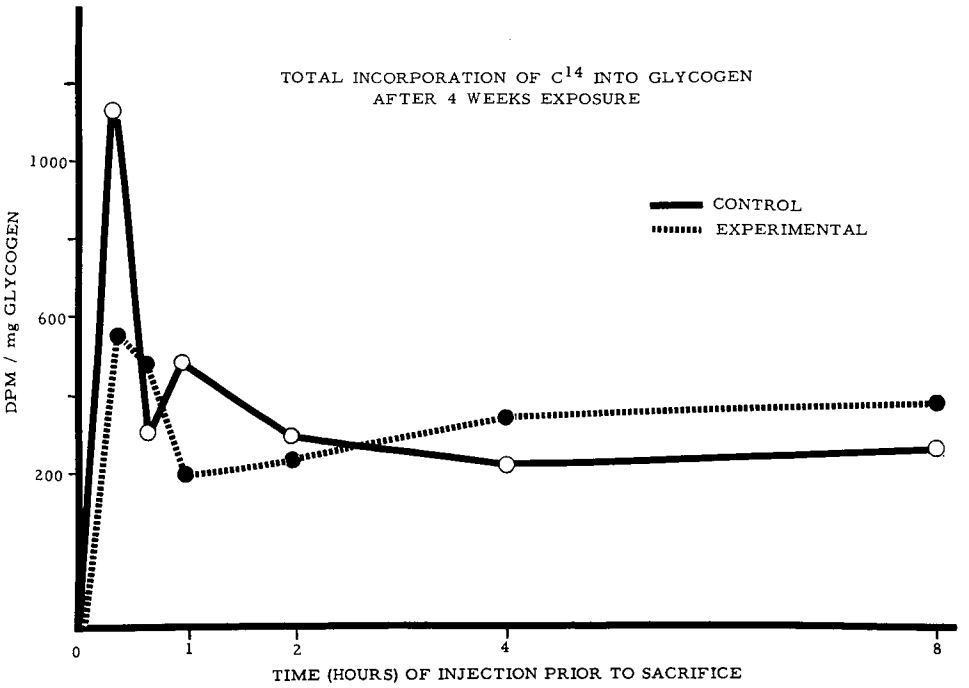


Figure 3

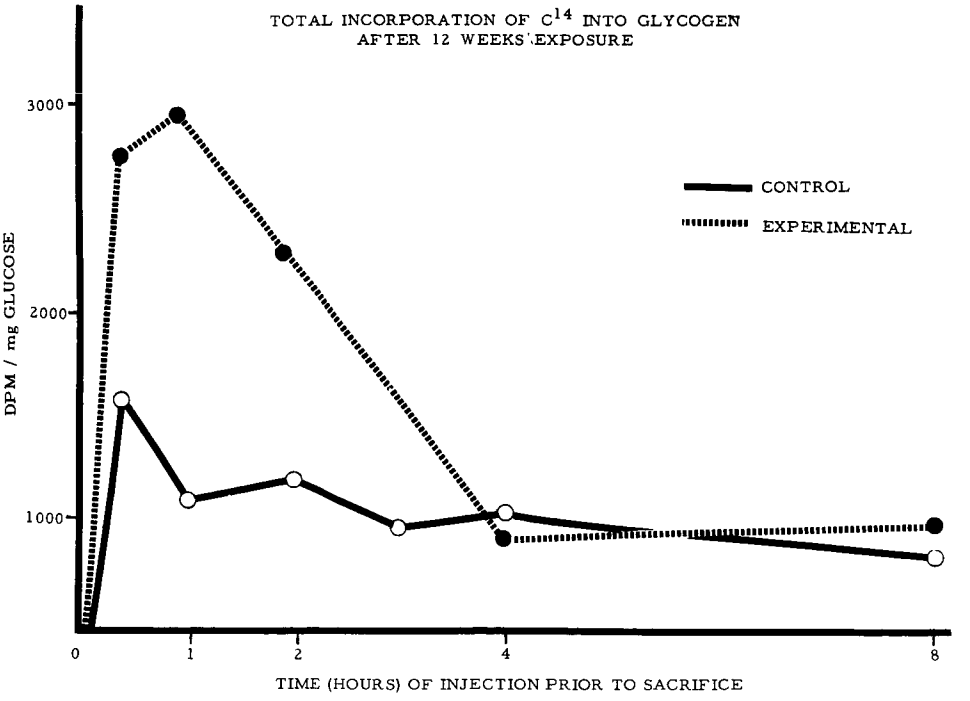


Figure 4

glycogen (expressed as net dpm/mg glucose) was almost the exact opposite of the 4-week data (Fig. 4).

It seemed important to look at other tissues as well since we already know that various tissues react somewhat differently to the experimental stress. After 4 weeks of exposure, both the heart and kidney glycogen had a higher specific activity throughout the first eight hours after injection of acetate-1-$C^{14}$. The specific activity peak occurred earlier and was greater in the case of the experimental animals' heart glycogen. This difference was even more pronounced in the kidney. After 12 weeks of exposure, the specific activity curves for both groups of animals were essentially superimposable both for the kidney and for the heart. Thus the heart and kidney responded differently to the experimental stress than did the liver and more accurately adapted to the environment.

c. $C^{14}$ Incorporation into Total Lipids

Plots of the turnover rates of $C^{14}$ from acetate in the total lipid pool of the liver (Figures 5 and 6) indicate that after 4 weeks of exposure, very little difference in total incorporation occurs until approximately eight hours after the injection

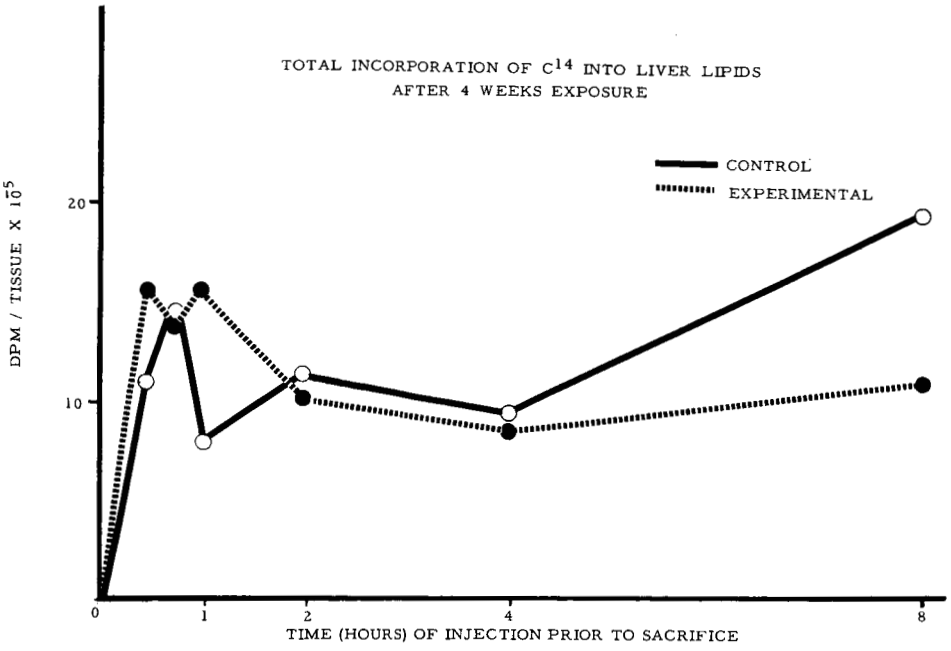


Figure 5


Figure 6

of radioacetate. At this time, the incorporation into the control animal lipids is greater. This was in agreement with results reported in earlier status reports. There is a suggestion that the peak of incorporation may occur earlier in the experimental animals , as indicated by specific activity data (Figure 6).

Similar plots for the 12 week exposed animals (Figures 7 and 8) indicate that after this period of exposure, the peak incorporation time was earlier in the livers of the experimental animals and both the specific activity and the total incorporation were greater in the experimental animals. These data are quite similar to the liver glycogen data. Furthermore, the turnover rate of the liver lipids appears to be faster in the experimental animals. This could result in a decrease in the total lipid in the liver and was therefore in agreement with the data concerning the lipid content of the liver (Table 6). Again there is a strong suggestion that the liver is attempting to compensate for the decrease in the rate of lipid synthesis observed after 4 weeks by incorporating more acetate into liver lipids. But perhaps the increased catabolic rate is still too great to allow complete normalization of the lipid content of the liver.

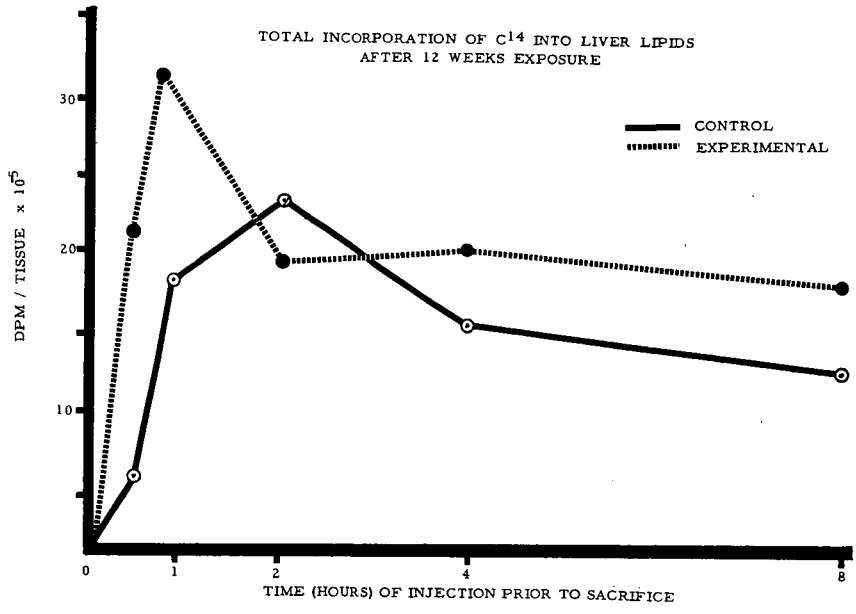


Figure 7

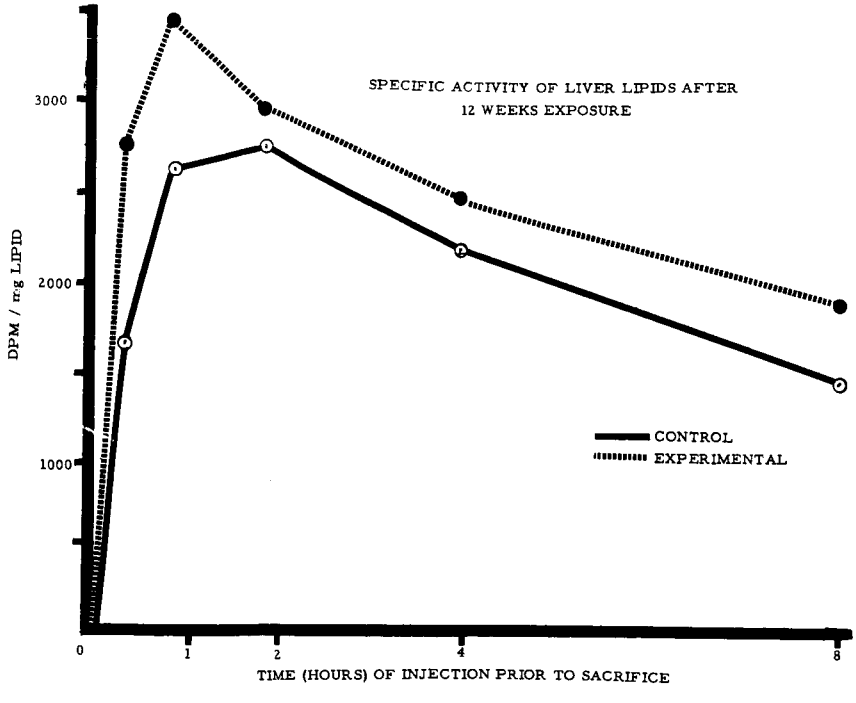


Figure 8

With respect to the heart lipids, there is remarkable consistency in both specific activity and total activity, as well as the amount of lipid (reported in section "a" above) when one compares the experimental animals with the control animals after both 4 and 12 weeks of exposure. The 12 week data are even more significant when one considers that the hearts were very significantly heavier in the experimental animal after 12 weeks of exposure but were essentially the same after only 4 and 8 weeks of exposure. This could be interpreted as indicating that the in situ metabolism of the hearts of the experimental animals was not significantly altered by the test environment but that the heart was required to work more strenuously to provide oxygen to and remove $CO_2$ from the cells in other tissues of the body. This interpretation is in agreement with the glycogen data and the blood gas data.

In the kidney, similar data indicate that after 4 weeks of exposure, the total incorporation and the turnover rate of $C^{14}$ from acetate was slower in the experimental animals but tended to return to normal after 12 weeks of exposure. These data are in excellent agreement with the lipid content data showing

that the kidney of the experimental animals had a little less fat after 4 weeks of exposure but returned to the comparable control level after 12 weeks.

d. Turnover Rates of Fractionated Lipid Pools

Fractionation of lipids of certain tissues on thin-layer chromatography and the turnover rates of these fractions are reported here for the liver and heart of animals exposed to the experimental environment for 4 weeks. Similar work on the kidney of these animals and liver, heart and kidney of the 12-week exposed animals is now in progress.

The bold differences in incorporation of $C^{14}$-acetate into six liver lipid fractions, expressed as a percent of the total radioactivity incorporated into liver lipid, are shown in a bar graph (Fig. 9). It is clear that the experimental animals incorporate somewhat less radioactivity in the triglyceride fraction than control animals (note particularly the 20 min., 1 hour and 4 hour data)and very little $C^{14}$-acetate into the unesterified fatty acids (UFA). These data are generally in agreement with data previously reported using silicic acid column chromatography for separation of liver lipids. On the other hand there

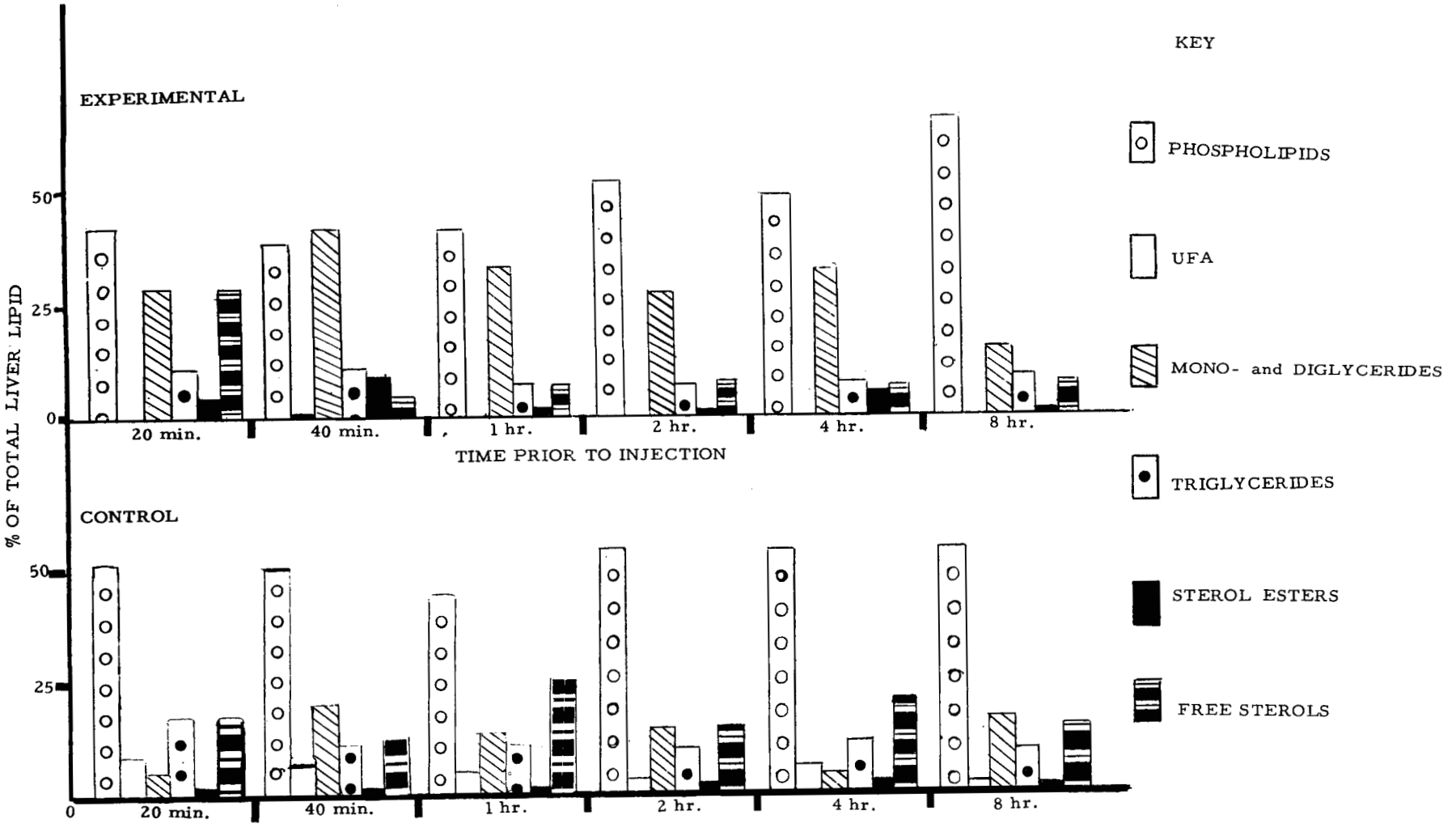


Figure 9. $C^{14}$ Incorporation into Liver Lipids (% of total $C^{14}$ incorporated into liver lipids)

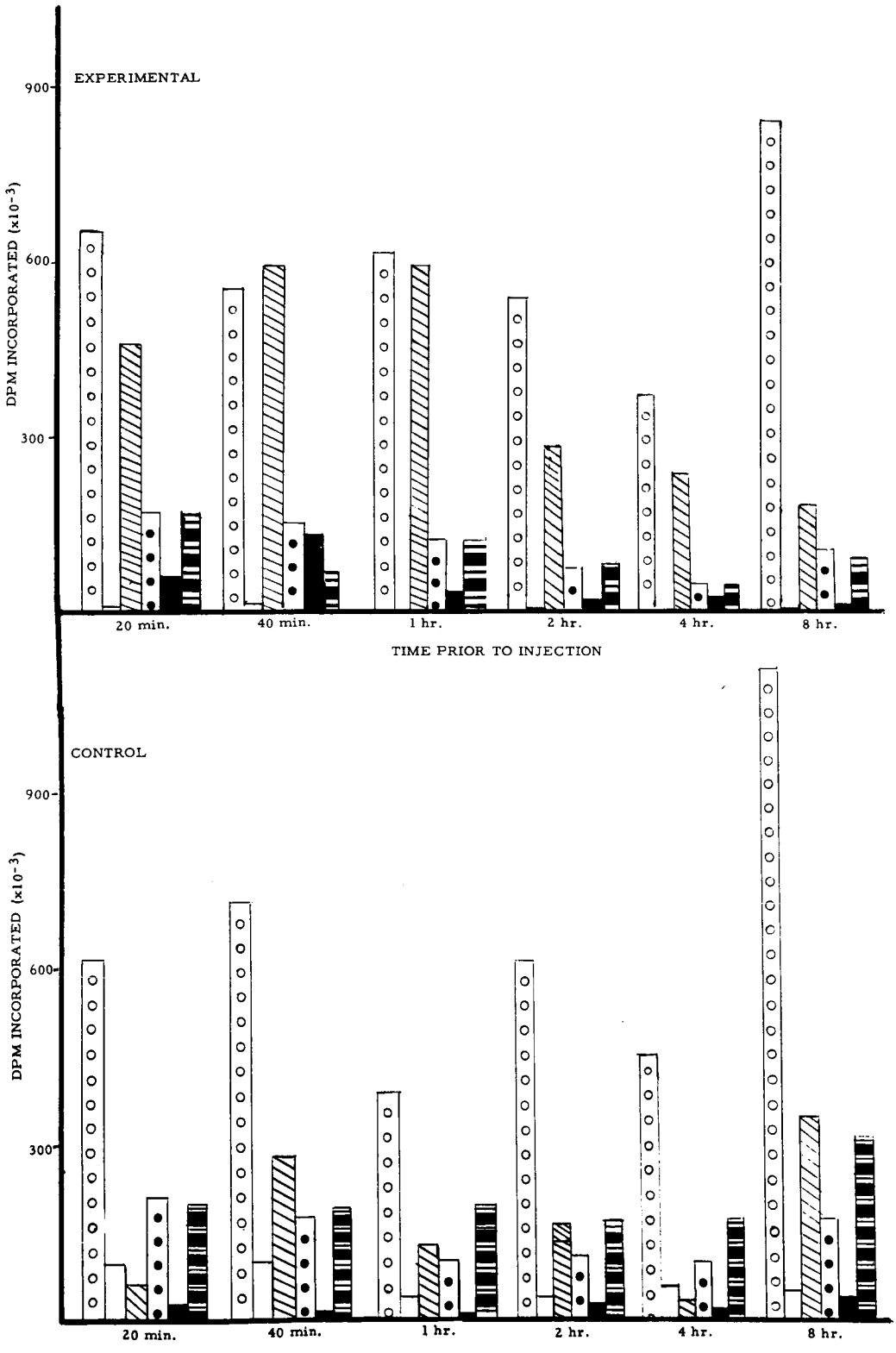


Figure 10. $C^{14}$ Incorporation into Liver Lipids (Absolute Values)

was a large increase in the relative amount of $C^{14}$ incorporated into the mono- and diglycerides in the experimental animals. In addition, the total amount of $C^{14}$ in the sterol esters was greater in the experimental animals while, in general, there was less in the free sterol fraction. It should also be noted that the specific activity of the free sterol fraction is consistently less in the experimental animals (Fig. 12).

Since the amount of lipid in the livers of the two groups of animals differed, the data are also expressed on an absolute basis as dpm incorporated into each fraction per liver (Fig. 10). The results were, in substance, the same.

Classically we consider the formation of mono- and diglycerides as intermediates in the synthesis of triglycerides from L-$\alpha$-glycerol phosphate and unesterified fatty acids, and free sterols and unesterified fatty acids as precursors of sterol esters. The sterol ester data are consistent since the formation of more sterol esters could lead to a reduction in free sterols and UFA, but the mono- and diglyceride data are not as easily explained. One possible explanation for the tremendous increase in radioactivity incorporation into mono- and diglyceride may be that, there exists, in the livers of the 4-week exposed animals,

a partial inhibition of the triglyceride synthesis and that triglyceride level is critical and its formation is controled by a feed-back mechanism. It seems desirable to hydrolyze the glycerides to determine whether the $C^{14}$ label is in the glycerol moiety or in the fatty acids. If the radiolabel is mostly in the glycerol, the fundamental problem may be a deficit of radioactive fatty acid. This does not seem too likely, however, since the $C^{14}$ activity of the phospholipids was not appreciably lower in the experimental animals (except for the 40 minute data) and each phospholipid molecule has one fatty acid and most contain two. Further, current evidence indicates that the syntheses of phospholipids and diglycerides share a common pathway until phosphatidic acid is formed and then the two pathways branch.

An in vitro experiment is planned to test the inherent capacity of the experimental animal livers to synthesize fatty acids from acetate and incorporate these acids into esterified products (glycerides and phospholipids).

The relative incorporation data for the heart lipids (Fig. 11) indicate that the major difference in this tissue was the definite increase in the amount of $C^{14}$ incorporated into the

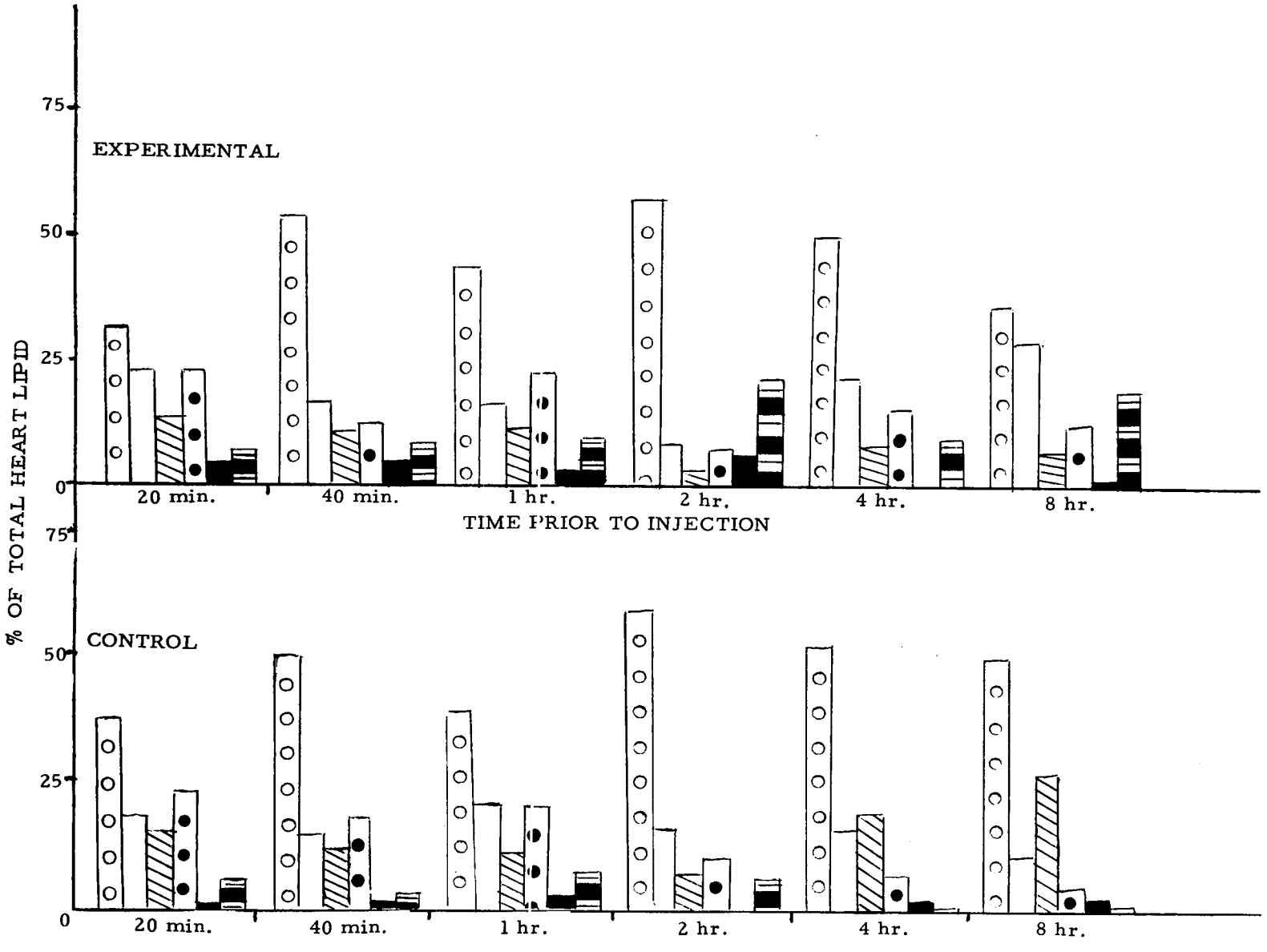


Figure 11. $C^{14}$ Incorporation into Heart Lipids (% of total $C^{14}$ incorporated into heart lipids)


**Figure ~~12~~ 13**

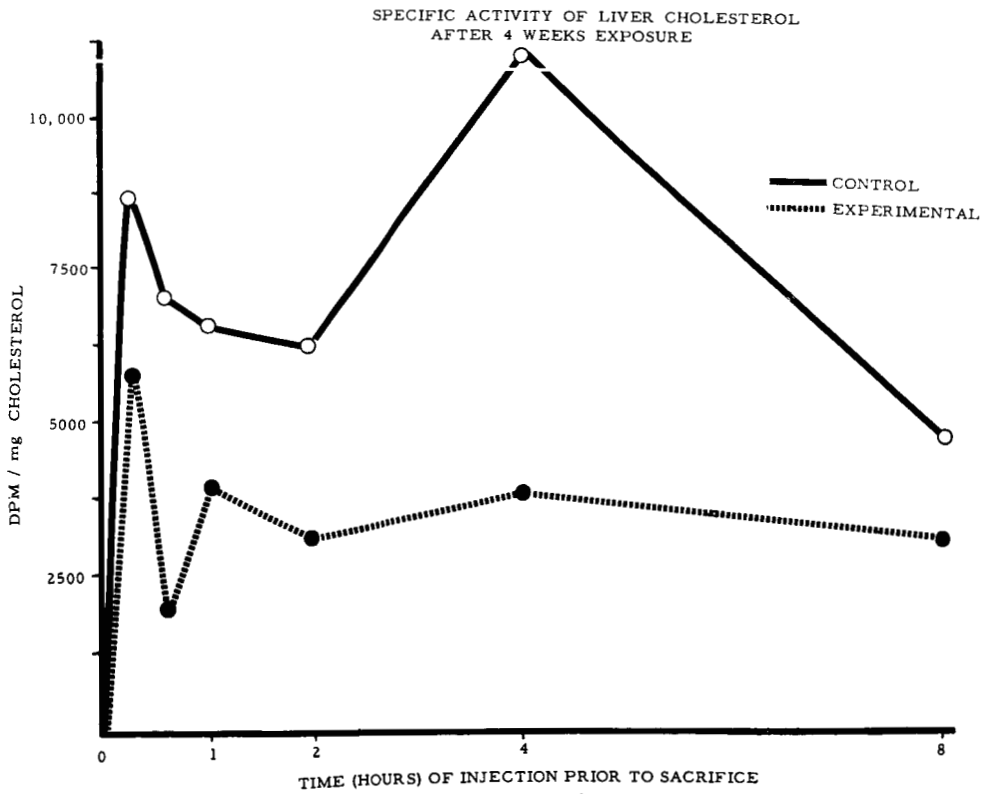


**Figure ~~13~~ 12**

experimental animal sterols (almost entirely cholesterol), both free and esterified. Additionally, the specific activity of the free sterols was greater in the experimental animal hearts (Fig. 13). This was true for the kidney as well.

It would seem, therefore, that after 4-weeks of exposure to the test environment, the livers of the experimental animals form more mono- and diglycerides and perhaps more esterified sterols while the heart formed more free and esterified sterols and formed the sterols faster than the control animals. It would be interesting, in the light of this evidence, to determine the blood cholesterol level and the deposition of sterol platelets in the aorta exiting the heart as an indication of whether prolonged exposure to the high oxygen-low pressure environment would increase the potential for atherosclerosis. This is particularly interesting since the tissues showed higher cholesterol levels after 4 weeks of exposure (Table 7). Although it is recognized that rats are relatively resistant to induced atherosclerosis (9), the danger of such a situation to astronauts may be significant.

e. Ketone Bodies

Although there was no difference in the concentration of acetone plus acetoacetate in the urine of experimental and control animals after twelve weeks of exposure, there was a marked difference in the incorporation of radioactivity (Table 8). The incorporation of carbon-14 into acetone and the acetone portion of acetoacetate was not greatly affected by the experimental environment but the radioactivity in the carboxyl carbon of acetoacetate was much higher in the urine of experimental animals.

TABLE 8

RADIOACTIVITY IN URINARY ACETOACETATE

| Weeks on Experiment | dpm x $10^{-3}$ in Acetone | | dpm x $10^{-3}$ in COOH | | Ratio $\frac{^{14}COOH}{Acetone}$ $-C^{14}$ | |
|---|---|---|---|---|---|---|
| | Control | Exptl | Control | Exptl | Control | Exptl |
| 4 | 34.6 | 49.4 | 92.2 | 464 | 3.08 | 9.57 |
| 8 | 136 | 170 | 286 | 2325 | 2.07 | 13.2 |
| 12 | 100 | 164 | 170 | 2640 | 1.77 | 15.4 |

Consequently, the ratio of carbon-14 in the carboxyl carbon compared to the acetone moiety is markedly greater in the experimental animals. Moreover, this ratio continues to increase the longer the animals are exposed to the experimental environment.

These data clearly show that either the experimental animals utilize a different pathway for acetoacetate biosynthesis or their source of substrate which becomes the carboxyl carbon has a much higher specific activity. The source of the carboxyl and alpha carbons of acetoacetate has been thought to be acetyl CoA, whether synthesis occurs by a direct deacylation of acetoacetyl CoA (10) or via β-hydroxy-β-methylglutaryl CoA (11). However, preliminary evidence (12) has been obtained which indicates that the carboxyl and alpha carbons of acetoacetate do not come directly from acetyl CoA but rather from protein bound acetate, perhaps analogous to the active acetate involved in fatty acid biosynthesis (13). In this connection, it should be noted that specific activity of liver lipids was higher in experimental animals than the controls during the initial period post-injection at 4 weeks (Fig. 6) and became even higher at 12 weeks (Fig. 8). The same general statement is true for total incorporation (Figs. 5 and 7). On the other hand, this increase in incorporation of radioactive carbon into the carboxyl carbon of acetoacetate and liver lipids does not appear to be correlated with the availability of coenzyme A since the concentration of coenzyme A was reduced at 4 weeks but returned to normal by 12 weeks (Section IV). Thus, the

major change in ketone body biosynthesis (acetoacetate) was apparently related to the method of synthesis rather than to the amount of synthesis.

## BIBLIOGRAPHY


1. Somogyi, M., J. Biol. Chem., 160:61, 1945

2. P. B. Hawk, B. L. Oser and W. H. Summerson, Practical Physiological Chemistry 13th Ed., McGraw-Hill Book Co., Inc., New York, 1954, p. 619

3. L. A. Greenberg and D. Lester, J. Biol. Chem. 154: 177, 1944

4. J. B. Allred, Manuscript in preparation

5. Harper, H. A., Review of Physiological Chemistry, 7th Ed., Lange Medical Publications, Los Altos, Calif., 1959, p. 151

6. N. Haugaard, M. E. Hess and H. Itskovitz, J. Biol. Chem. 227:605, 1957

7. Status Report, NsG 300-63-01, 31 October 1964

8. Status Report, NsG 300-63-01, 30 April 1964

9. Clarkson, T. B., Adv. in Lipid Res., 1:211, 1963

10. G. I. Drummond, and J. R. Stern, J. Biol. Chem., 235:318, 1960

11. F. Lynen, U. Henning, C. Bublitz, B. Sorbo and L. Kroplin-Rueff, Biochem. Z., 330:269, 1958

12. Lynen, F., Redoxfunktionen Cytoplasmatischer Strukturen Symposium, unter der Leitung von Th. Bucher, Marbug/Lahn, Gemeinsametagung der Deutschen Gesellschaft fur Physiologische Chemie und der Osterreichischen Biochemischen Gesellschaft, Wien, September 26-29, 1962

13. P. W. Magerus, A. W. Alberts and P. Roy Vagelos, Proc. Nat'l Acad. Sci., 51:1231, 1964